Environmental Health Perspectives
Vol. 13, pp. 37–42, 1976

Investigation of the Mechanism of Action of Atmospheric Pollutants on the Central Nervous System and Comparative Evaluation of Methods of Study

by A. I. Bokina,* N. D. Eksler,* A. D. Semenenko,* and R. V. Merkur'yeva*

Some aspects of the mechanism of action of atmospheric pollutants (acetone, benzene, ammonia, formaldehyde, and ozone) on the central nervous system were studied by using methods of functional electroencephalography (analysis of the readjustment reaction to a rhythmic light stimulus, evoked potentials of the cerebral cortex, and determination of the photometrazol thresholds). Effects of the compounds were determined for the various structures of the cerebral cortex of experimental animals. The most sensitive structures were those which were first to associate in the reaction to toxic agents (the corticomedial nucleus of the amygdaloid complex and the olfactory bulb). EEG indices were observed which were indicative of an adverse effect (epileptoid activity in the most sensitive formations of the brain and a stable generalized stress rhythm in the neocortex and in the limbic ascending reticular system). During long-term action of toxic materials at low concentrations, changes were observed in the parameters of the primary and secondary responses of the visual evoked potential which were indicative of a disturbance of the cortical inhibition processes. This can be considered one effect of atmospheric pollutants at low concentrations. Problems of the comparative sensitivity of the various methods of studying the central nervous system are being investigated with a single compound: carbon bisulfide. In human experiments concerned with the fine coordination of measured movements such as writing and the solution of arithmetic problems, the subject-operator observed that repeated inhalation of subsensory concentrations of carbon bisulfide (0.08 mg/m^3 level) disturbs the rate of execution of assigned motor processes. In tests with rats with developed instrument conditioned reflexes, it was shown that entire behavioral acts deteriorate under the effect of the same carbon bisulfide concentration. In tests on rabbits, simultaneous neurophysiological and neurochemical analyses were performed on the changes in the functional state of the central nervous system under chronic exposure to carbon bisulfide at various concentrations.

Pursuant to the U.S.–U.S.S.R. agreement on medical aspects of environmental health, the program of joint studies calls for neurophysiological analysis of the effect of air pollutants on the functional state of the brain and a comparative evaluation of the electrophysiological methods used in the U.S.S.R. and the U.S. to assess the toxic effect of chemical substances.

The purpose of the research along the first of these lines was to clarify the mechanism by which certain air pollutants (acetone, benzene, ammonia, formaldehyde, ozone) affect the CNS through brief

*A. N. Sysin Institute of General and Communal Hygiene of the U.S.S.R. Academy of Medical Sciences, Moscow, U.S.S.R.

or prolonged exposure in high or low concentrations, to identify the most sensitive parts of the brain, and to find electroencephalographic indicators of the adverse effect of air pollutants.

EEG Indicators of Effect of Pollutants

The methods of functional electroencephalography, being the most sensitive and informative, were used in attacking this problem; areas of study were the rhythm change reaction, induced potentials of the cerebral cortex, and the photometrazol thresholds. Experimental research on rabbits dealt with the overall electrical activity of various brain structures which account for the sensory response to olfactory stimuli (olfactory bulb, pyriform cortex) and with the systems which organize adaptive-behavioral reactions (amygdala, hippocampus, reticular formation of the brain stem). This approach is new in health research.

Together with measured stimulation by chemical substances, various afferent stimuli were used in the experiments: sound (frequency of 500 Hz, volume of 20 and 80 dB), light in rhythmic or aperiodic flashes (1.4 joules), stimulation of the conducting pathways of the olfactory brain by rectangular electrical pulses.

The results indicate an extremely high selectivity in the impact of the toxic agents studied. The character of the electroencephalographic reactions and the sequence of the response of brain structures to the action of the substances studied whether in low or in high concentrations indicate that the most sensitive structures, responding before others to the influence of toxic agents with pathological reactions, are the olfactory analyzer structures (corticomedial nucleus of the amygdaloid complex).

It has been found that chemical substances, when used for brief (10 sec) periods, produce two types of reactions, depending on the concentration level of the exposure: a nonspecific reaction of orientation and exploration and a specific reaction. The former is characterized by the appearance in the neocortex of individual flashes of activity and by quickened respiration, while no change in activity is noted in the olfactory analyzer structures. The specific reaction is characterized by depression of high frequency-induced activity in the olfactory structures (Fig. 1).

Concentrations of chemical substances eliciting an orienting-exploratory reaction are evaluated by the central nervous system as undifferentiated stimuli, since the form of response to these stimuli coincides with the electrographic reaction to other sensory stimuli (light, sound).

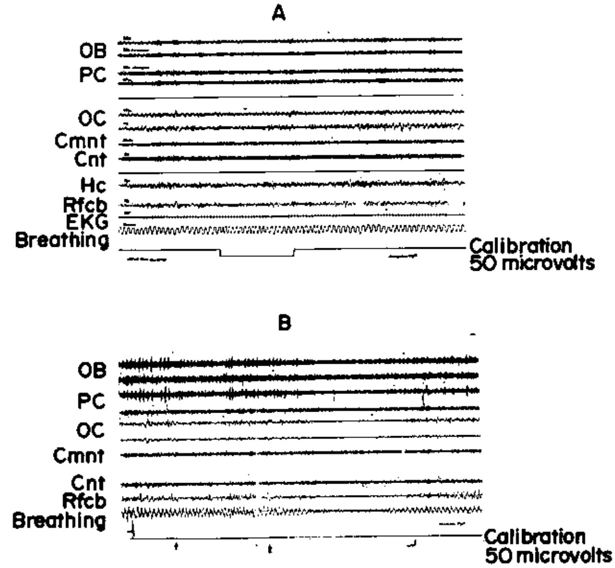

FIGURE 1. Example of (*A*) orienting-exploratory and (*B*) specific electroencephalographic reaction to the effect of formaldehyde at the following concentrations: (*A*) 3.7 mg/m^3 and (*B*) 12.6 mg/m^3. Designations of structures: OB = olfactory bulb, PC = pyriform cortex, Cmnt = corticomedial nucleus of a tonsil; Cnt = central nucleus of a tonsil; Hc = hippocampus, Rfcb = reticular formation of the brain; EKG = electrocardiogram.

The specific reaction must be categorized as adaptive (*1*), since it is a biologically significant reaction of the organism.

It should be noted that the changes in the electroencephalograms after a 10-sec exposure to chemical substances are brief, with a rapid return to the initial functional state of the brain.

When exposure to such substances is prolonged for up to 20 min, however, basic alterations occur in the electrical activity of the brain structures studied. A persistent stress rhythm develops in the cortex and limbic reticular structures which correlates in some animals with an avoidance reaction and indicates a change toward excitement in the animals' functional state (*2*). A protracted, generalized stress rhythm may be taken as an indicator of an adverse effect of odorous toxic substances on the CNS.

Thus, an adverse biological significance in electroencephalographic reactions to the influence of chemical substances in cases of brief exposure can be identified only when the substances are used in concentrations at the specific reaction level or, for lower concentrations, when the time of exposure is increased to 20 min.

A study of sensitization during repeated exposure reveals that, when animals have been subjected to an initial exposure to high concentrations of chemical substances, the use of those same substances in low concentrations, combined with a functional load (rhythmic light), provokes paroxysmal activity in the olfactory analyzer structures (Fig. 2). Paroxysmal forms of activity in the olfactory analyzer structures are definite signs of an adverse effect of the toxic substances on the central nervous system.

The evoked potentials of the cerebral cortex were studied in conditions of chronic exposure to the above-mentioned air pollutants

Figure 3 shows the dynamics of changes in evoked potential during chronic exposure to formaldehyde in concentrations of 0.1 mg/m^3 and to ozone in concentrations of 0.05 mg/m^3. A fall in amplitude of the primary response of the evoked potential, a slow negative wave, and a delayed response, as well as a decrease in the duration of the slow negative wave towards the end of exposure was observed. From the physiological viewpoint, this shift may be deemed unfavorable, since neurophysiological analysis of the results indicates a deterioration of the cortical inhibitory processes (*3, 4*). The drop in overall EEG amplitude of all the brain structures studied and the behavioral reactivity observed in the animals when so exposed suggest that the indicated concentrations, along with application of functional burdens, should not be categorized as having no effect on the organism.

It may be assumed that the changes in induced potential discovered in the prolonged experiment reflect an initial prepathological reaction of the brain, since the process of change in induced potentials suggests a possible manifestation of the brain's paroxysmal activity during longer exposures or under the effect of higher concentrations. This result would tend to support the concept of a general mechanism by which toxic substances, when used at high concentrations, or at low concentrations for a prolonged period, act upon the synaptic organization of the brain.

The results also indicate that the effect on functions of the CNS of the investigated compounds, though they differ in chemical structure and physical and chemical properties, is alike in principle (although in different time frames). This result fits in well with the considerable data in the health literature on the simple additive effect of exposure to a complex of toxic substances on other functional systems.

Analysis of the results shows that deviations in the parameters of the rhythm change reaction and

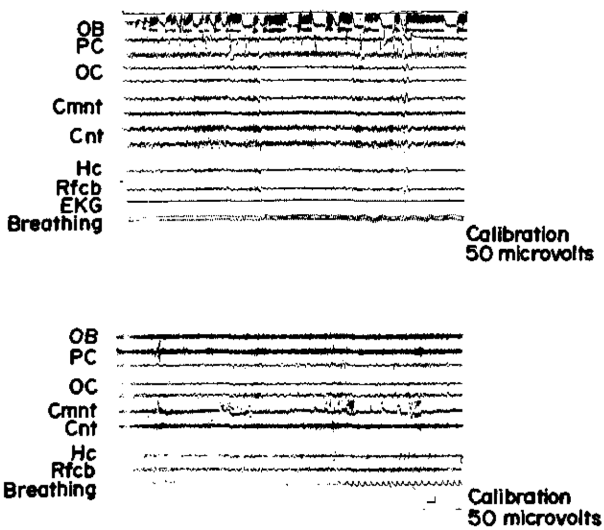

FIGURE 2. Paroxysmal activity in the structures of the olfactory analyzer (olfactory bulb and cortico-medial nucleus of tonsil) under the effect of ozone at a concentration of 0.03 mg/m^3 after its preliminary use at a concentration of 4–6 mg/m^3. Designations and scale same as in Fig. 1.

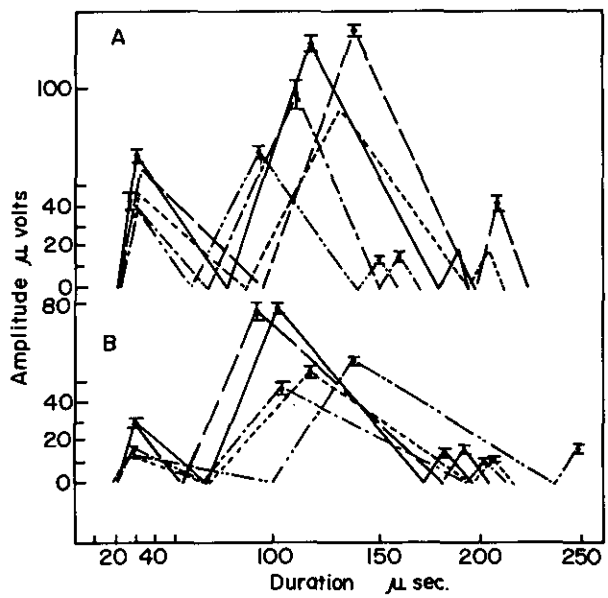

FIGURE 3. Dynamics of the change in the parameters of the primary and secondary responses of the evoked potential of the optic cortex during the course of 1.5 month continuous exposure to (*A*) formaldehyde at a concentration of 0.1 mg/m^3 and (*B*) ozone at a concentration of 0.05 mg/m^3.

alterations in the time – amplitude characteristics of the initial response and secondary reactions of induced potentials, indicative of the adverse effect of air pollutants, may figure among the essential criteria used in the setting of standards.

Evaluation of EEG Methods

The second goal of our research was a comparative evaluation, on the basis of a single substance—carbon bisulfide—of the sensitivity of the electroencephalographic methods use.

CS_2 was chosen because of its great industrial significance and the lack of data on its effect in low concentrations.

The carbon bisulfide was identified by colorimetric means, based on the reaction of CS_2 with diethylamine and then copper acetate, yielding diethyl dithiocarbamate. The study took into account the relationship between the concentration of the substance and it's effect.

As we know, one of the basic integral forms of an organism's vital activity is the motor function, which endows man's behavior in his environment with a dynamic character.

In this regard, a methodological approach was chosen which made it possible to study the integral reaction of the organism and directly to define the efficiency and concomitant fine coordination of human movements in conditions of exposure to chemical air pollutants.

The scientific basis for the study of the behavioral reactions of man and animals was the concept of the Soviet school of physiologists (2) on self-regulating functional systems of the organism with feedback on the results of the action.

With the aid of a physiological model based on teaching the organism to choose its actions, we studied motor coordination by having a subject execute precisely graduated rapid hand movements, imitating separate fragments of common work processes.

The subjects (20 persons) worked in two modes. In the first work mode, the subject was required to carry out, pursuant to instructions, a program of differentiated movements at the pace of emitted sound (or light) signals of varying intensity. Upon emission of the signals, the subject was to press quickly with his fingers, using a specific force and with a given frequency, on the discs of a sensitive sensor, under the control of the muscular-articular sense alone.

In the second work mode, a further instruction was given: when the rhythmic sound stimuli terminate, reproduce the program of movements from memory to the same time intervals.

The ability of a person to differentiate the muscular force of angular motions of the arms and legs was also studied. The active selection of actions was used for this purpose, with the subjects themselves choosing the initial size of all movements in the program and trying to maintain their chosen parameters throughtout the experiment.

In addition, the work program included the performance of a complex act such as the process of writing, requiring the coordination of several bodily systems, and the solving of arithmetic problems.

A series of indicators was recorded: the length of the latent period, the amplitude of muscular and angular arm movements, the speed and precision with which the assigned program was carried out, the impairment of differentiated motor reactions, and the speed and extent to their restoration. The errors made by the subjects as they carried out the program were analyzed.

The chemical irritant used was carbon bisulfide vapor, administered in spurts, in concentrations that could not be detected by smell, for 10–15 min during the experiment.

For quantitative analysis of the biological processes, a multichannel integrator with graphic recording of impulse readings and a magnetic recording system with subsequent automatic numerical analysis of the results were used.

Repeated inhalation of carbon bisulfide in subsensory concentrations (at the level of 0.08 mg/m^3) leads to an impairment in the speed of execution and the coordination of certain motor processes.

These changes suggest a maladjustment in the body's motor coordination system and a diminished control over the results of one's actions when air pollutants are affecting the organism.

Behavior reactions at low concentrations of carbon bisulfide vapor were studied in parallel on 50 rats with electrodes implanted in the cortical structures of their brains. The controlled experiment model which we had developed, facilitating the study of complex behavioral reactions in experimental animals, was used.

Instrumental reflexes and extrapolative reflexes of varied complexity, which are the foundations of the organism's ability to use past experience as a basis for purposeful forms of behavior, were developed in the animals. While recording various data on behavioral reactions (reaction time, strength of muscular pressure on pedals, number of mistakes in executing the program), we also determined basic parameters of the elcetrical activity of the brain: the latent period of the rhythm change reaction, the index of assimilation of light stimuli, and the amplitude-frequency description of the background EEG.

Low concentrations of carbon bisulfide vapors were administered during the research on the rats. The chemical irritant was administered in spurts and continuously for from 5 min to 2 hr.

The accumulated experimental data indicate that, with repeated exposure to carbon bisulfide (0.08 mg/m^3), rats, like humans, show a maladjustment in the body's motor coordination system. A deterioration of integral behavioral acts results which is reflected first of all in an impaired ability to choose the most suitable variant for achieving one's goals.

In order to evaluate the state of cortical inhibition, we used the method for studying the evoked potentials of the cerebral cortex in conjunction with the effect of low CS_2 concentrations (0.2 mg/m^3). This method was used in studies on rabbits with electrodes implanted in the area of the vision cortex.

Constantan electrodes (d = 0.8 mm) were used to record the evoked potentials. Light pulses (flash energy 1.4 joules, duration 1.2 msec) were used to evoke the potentials. A UBP-1 alternating current booster was used to amplify the induced potentials. The ATAS-201 computer was used for registering evoked potentials (operating in an averaging mode with 50 realizations each).

The following components of the evoked potentials were analyzed: initial response, slow negative wave, delayed response (latency, maximum amplitude of individual phases, steepness of their rise, and duration of evoked potential parameters).

The protracted priming of the rabbits with carbon bisulfide (1.5 months, 24 hr per day) took place in 200-liter inhalation chambers with the air being changed 30 times. The control group of animals was placed in similar chambers through which air cleansed of dust and gas contaminants was circulated.

During the first 4 weeks of chronic exposure, none of the evoked potential parameters differed from the control or background parameters. During the sixth week of exposure to CS_2 at a 0.2 mg/m^3 concentration, a statistically significant ($p < 0.05$) increase occurred in the amplitude of the secondary positive deviation (Table 1).

Analysis of the mean values of this parameter shows that a progressive increase takes place during the course of the chronic action with carbon bisulfide.

A CS_2 concentration of 0.2 mg/m^3 can be considered a threshold level for the given species of animal.

It was deemed advisable to conduct a biochemical study of the neuraminic acid metabolism in the brain tissue of rabbits which had been exposed to carbon bisulfide in view of the important role played by glycoproteids containing neuraminic acid in executing the functions of the central nervous system.

With this in mind, the Warren method (5) was used for determining the neuraminic acid content

Table 1. Evoked potential parameters of the optic cortex during chronic exposure of rabbits to carbon bisulfide at a concentration of 0.2 mg/m^3 (average value for six animals).

Effect period	Primary response: Latent period of + phase, msec	Primary response: Latent period of − phase, msec	Primary response: Amplitude, μV	Secondary positive deviation: Latent period msec	Secondary positive deviation: Amplitude μV	Slow negative wave, amplitude, μV	Late response, amplitude μV
Background	27.7 ± 2.0	38.0 ± 2.7	56.2 ± 1.1	63.2 ± 2.7	51.7 ± 33.6	95.8 ± 20.3	15.5 ± 16.8
2nd Week	29.9 ± 0.9	39.1 ± 1.8	66.5 ± 10.5	67.0 ± 1.6	97.1 ± 24.1	123.0 ± 21.5	41.0 ± 30.6
4th Week	29.2 ± 2.2	37.0 ± 2.7	43.0 ± 11.9	65.0 ± 4.2	116.5 ± 62.3	111.7 ± 26.3	18.0 ± 5.4
6th Week	30.5 ± 0.8	38.7 ± 3.6	124.6 ± 70.4	65.0 ± 2.8	158.2 ± 101.0[a]	231.5 ± 110.5	26.3 ± 15.9

[a]$p < 0.05$.

in brain tissue. The activity of aldolase (an enzyme which breaks down free neuraminic acid into *n*-acetylmannosamine and pyroracemic acid) on neuraminic acid was also determined.

Results of the biochemical study show that carbon bisulfide has a significant effect on the metabolism of the sial-containing glycoproteins in the brain tissue of test rabbits in comparison to the animals in the control group. The most significant changes under these conditions were noted in the aldolase activity on neuraminic acid, whose level in some of the experimental animals was reduced to 7–10%. This is lower by a factor of 1.5–2 times than its value for the brain tissue of the rabbits in the control group. The extent of changes in the neuraminic acid content in brain tissue was less.

Comparison of the results from the study of neuraminic acid level and activity of its breakdown enzyme in the brain tissue of experimental animals showed that the disturbance of the metabolism of this substance during the effect of carbon bisulfide may not only be related to a given catabolic phase, but also to the enzymatic splitting reaction of free neuraminic acid from a glycoproteid molecule in the presence of neuraminidase. It can also affect the biosynthesis of sialic acid. This suggestion requires additional study.

Comparison of the neurochemical and electrophysiologic data made it possible to determine the correspondence between the direction and degree of change in the amplitudes of the secondary positive deviations of the evoked potentials and the disturbance in the activity of neuraminic acid aldolase in the brain tissue of experimental animals. The most marked progressive increase in electrophysical deviations associated with individual animals was accompanied by the most significant reduction of neuraminic acid aldolase activity, and, conversely, the absence of changes in the electrophysiological acitivity of the brain was observed to occur simultaneously with a normal state of neuraminic acid metabolism in brain tissue.

The parallel observed by us between the electrophysiological and the neurochemical study data permits the suggestion that the reduction in the enzymatic splitting of free neuraminic acid may be one of the probable manifestations of the changes in the electrophysiological activity of the brain on the neuron level. The biochemical reason for this phenomenon may be related to the protective function of neuraminic acid on the neuron surface and to the necessity of protecting it in the cellular membrane structures in response to the toxic action of carbon bisulfide.

Thus, functional EEG methods permit us to make judgements as to the changes in the overall functional state of CNS, to obtain a differentiated characteristic for the state and sensitivity of the individual structures of the brain, to determine the specificity of their reactions, and to record the anomalous forms of activity.

Functional EEG methods (rhythm readjustment reaction and evoked potentials) have been used and can be used in animal experiments to study the action of chemical substances on an organism (adaptation processes, sensitization, and other general biological regularities associated with the toxic effect of air pollutants on the organism).

Methods of functional electroencephalography which are being used in studies on humans (particularly the rhythm readjustment reaction) are sensitive and informative. Nevertheless, studies on the mechanism responsible for the effect of environmental pollutants on the central nervous system showed that we must have a 20-min chemical factor effect in order to get significant changes.

The methods of studying behavorial reaction are integral and adequately sensitive and are most suitable for the evaluation of chemical compounds. It should be noted that the effect of chemical substances at low concentrations is most marked against the background of forming, but not to the point of automatic, specific behavioral reactions.

In conclusion, it should be noted that combining the methods of functional electroencephalography and behavioral toxicology is most productive. Their combination makes it possible to make use of the advantages of each of these and to make a complete study of the toxic effect of atmospheric pollutants on an organism.

REFERENCES

1. Sokolov, Ye. N. Neuron mechanisms of the orientation reflex. In: Nevronnvi Mekhanismy Orientirovotsiogo Refleksa (Neuron Mechanisms of the Orientation Reflex), Moscow, 1970, p.3.
2. Anokhin, P. K. Electroencephalographic analysis of the cortical–subcortical relationships for positive and negative conditioned reactions. In: Vysshaya Nervnaya Deyatel' nost (Higher Nervous Activity), Moscow, 1968, p. 86.
3. Kondrat'eva, I. N. Inhibition in the systems of the neurons of the visual area of the cortex. Zh. Vyssh. Nervn. Deyatel. im. I. P. Pavlova 14: 1069 (1964).
4. Skrebitskyi, V. G., and Voronin, L. L. Intracellular study of the electrical activity of the neurons of the visual cortex of an unanesthetized rabbit. Zh. Vyssh. Nervn. Deyatel. im. I. P. Pavlova 16: 864 (1966).
5. Warren, L. The thiobarbituric acid assay of sialic acids. J. Biol. Chem. 234: 1971 (1959).